Acute Thyroid Hormone Withdrawal in Athyreotic Patients Results in a State of Insulin Resistance

Gabriela Brenta,¹ Francesco S. Celi,² Mario Pisarev,³ Marta Schnitman,⁴ Isaac Sinay,⁵ and Pablo Arias⁶

Dear Editor:

It is well known that thyroid hormones are important regulators of energy balance and intermediate metabolism. With regard to glucose homeostasis, hyperthyroidism is associated with a state of insulin resistance due to increased hepatic gluconeogenesis (1). In hypothyroidism however, despite the fact that hepatic gluconeogenesis has been described as unchanged (2) or decreased (3,4), experiments in animal models have demonstrated a state of insulin resistance both in the adipocyte and in the skeletal muscle (5,6). In humans the literature referring to the connection between thyroid dysfunction and insulin resistance is not consistent; this is in part due to the complex and redundant mechanisms regulating glucose homeostasis, but also to the use of different methods and indexes to assess the insulin sensitivity (indexes derived by the fasting insulin and glucose levels, intravenous [IV] or oral glucose tolerance test, euglycemic hyperinsulinemic clamps, local infusion of insulin and glucose in isolated limbs, examination *in vitro* of adipocytes, etc.). Specifically, only few studies have evaluated the insulin sensitivity in hypothyroidism and the results are controversial, some (7–12), but not all (13–16) have found that thyroid hormone deficit leads to insulin resistance secondary to decreased glucose disposal. On the other hand, while overt hyperthyroidism has been clearly associated with insulin resistance (1), the data on insulin action in subclinical hyperthyroidism is limited and controversial (17,18).

The use of thyroid hormone suppressive therapy for thyroid cancer and its withdrawal before diagnostic or therapeutic intervention offers a unique model to assess the effects of controlled manipulation of circulating thyroid hormones on target pathways, in this case glucose homeostasis.

We hypothesized that the lack of thyroid hormones leads to an insulin-resistant state due to a decrease in glucose disposal in skeletal muscle and adipose tissue. Considering the critical impact of insulin resistance on cardiovascular risk (19), we

have explored the changes in insulin sensitivity in relation to changes in thyroid hormone homeostasis both under the subclinical hyperthyroid and overt acute hypothyroid status in thyroid cancer patients undergoing thyroid hormone withdrawal.

Six women (age 42 ± 11 years, body mass index [BMI] 24 ± 2 kg/m²) that underwent total thyroidectomy for differentiated thyroid cancer were included in the study group. Eight healthy subjects (six women and two men, age 37 ± 2 years, BMI 23 ± 2 kg/m²) served as controls. The characteristics of the thyroid cancer patients are reported in Table 1. All the volunteers had a normal response to a standard 75 g oral glucose tolerance test. No patient had clinical evidence of metastatic disease as assessed by suppressed thyroglobulin and neck ultrasound. The average dose of levothyroxine taken orally every morning for TSH suppression was 169 ± 34 µg/d. Four patients were studied under TSH suppression with levothyroxine (sub HYPERT). Due to the fact that two volunteers dropped out after the initial evaluation, two new volunteers were recruited to replace them in the following phases of the study. This group of four patients were studied 1 week after thyroid hormone withdrawal (EUTHYR) and again, after 6 weeks of levothyroxine withdrawal in a hypothyroid state (HYPO). Eight euthyroid subjects served as controls (CON). None of the subjects was critically ill or taking any medication that might influence glucose metabolism. All study participants provided their informed consent for the study which was approved by the Institutional Review Board and Ethics Committee.

Free thyroxine (T₄), total triiodothyronine (T₃), and thyrotropin (TSH) were measured by chemiluminescence (DPC; Immulite, Los Angeles, CA); normal range for free T₄, 0.8–1.9 ng/dL; intra- and inter-assay coefficient of variation were 5.8% and 6.7%, respectively; normal range for T₃, 0.8–2 ng/mL; intra- and inter-assay coefficient of variation were 5.8% and 6.6%, respectively; normal range for TSH was 0.3–5.0 µU/mL; intra- and inter-assay coefficient of variation were

¹Department of Endocrinology and Metabolism, Cesar Milstein Hospital, Buenos Aires, Argentina.

²Clinical Endocrinology Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland.

³Department of Biochemistry, Buenos Aires University Medical School; Department of Radiobiology, National Commission of Atomic Energy (CNEA); Buenos Aires, Argentina.

⁴Department of Endocrinology and Metabolism, Cesar Milstein Hospital, Buenos Aires, Argentina.

⁵Diabetes Section, Cardiovascular Institute of Buenos Aires, Buenos Aires, Argentina.

⁶Department of Physiology, Buenos Aires University Medical School, Buenos Aires, Argentina.

TABLE 1. CLINICAL AND ANTHROPOMETRIC DATA OF THE STUDIED POPULATION

Patient	Age (years)	BMI (kg/m ²)	Cancer histology	T ₄ dose (μg)	Other treatments
1	35	24	Follicular	200	
2	65	21	Papillary	125	Alendronate, calcium, vitamin D
3	37	25	Follicular	200	
4	32	24	Papillary	150	
5	44	26	Papillary	200	
6	43	24	Papillary	150	Alprazolam

BMI, body mass index; T₄, thyroxine.

2.3% and 3%, respectively. Glucose levels were measured in serum using commercial available assays (Roche Diagnostics, Mannheim, Germany).

Both patients (HYPER, EUTHYR, and HYPO) and controls were admitted to the Endocrine Division in the morning after a 12-hour overnight fast. Insulin sensitivity was assessed by using the short IV insulin tolerance test (20). Briefly, an indwelling catheter was placed in an antecubital vein; baseline glucose levels were obtained by averaging the values of the samples obtained at times -5 minutes and 0 minutes, then, 0.1 IU/kg of regular insulin (Actrapid HM; Novo Nordisk, Bagsvaerd, Denmark) was administered as a bolus in 10 seconds, and blood samples were collected every 3 minutes. The short IV insulin tolerance test was stopped after 15 minutes to prevent hypoglycemia and the initiation of counter-regulatory mechanisms. Insulin sensitivity is expressed as Kitt (=69.3/t_{1/2}), which reflects the rate of glucose disappearance from the plasmatic compartment. The decremental area under the curve (expressed as mg/dL×15 minutes) was also calculated by the trapezoid rule. For the statistical analysis data, expressed as means ± SD, were tested for normality using the Kolmogorov-Smirnoff approach. Differences among the sub HYPER, EUTHYR, HYPO, and CON groups were tested for statistical significance using the Kruskal-Wallis test, followed by the Dunn *post hoc* test. A paired Student *t* test was used to compare only the EUTHYR and HYPO data. An α error <0.05 was considered statistically significant. Data handling and statistical analysis were both performed using Prism 4 for Windows (Version 4.0, 2003; GraphPad Software Inc, San Diego, CA).

Thyroid hormone and TSH levels are reported in Table 2. As expected, differences between the sub HYPER and HYPO groups were statistically significant (free T₄ sub HYPER 1.9 ± 0.18 ng/dL vs. HYPO 0.24 ± 0.2 ng/dL, $p < 0.01$; TSH sub HYPER group 0.15 ± 0.0 μU/mL vs. HYPO 34.7 ± 17.6 μU/mL, $p < 0.01$). T₃ levels were different between CON 1.07 ± 0.1 ng/mL and HYPO 0.4 ± 0.2 ng/mL, $p < 0.01$.

All study volunteers tolerated the insulin tolerance test well and no adverse event was recorded. The response to the insulin bolus of each of the four groups is reported in Fig. 1. While baseline and post-insulin plasma glucose concentrations were similar among the groups, a significant difference between the CON (423.2 ± 48.12) and HYPO groups (268.4 ± 54.81) was observed in the decremental area under the curve (mg/dL glucose×15/min, $p < 0.05$) (Fig. 2). While no signifi-

TABLE 2. THYROID HORMONE VALUES

	Sub HYPER	EUTHYR	HYPO	CON
TSH μU/mL	0.15	1.69 ± 1.5	34.7 ± 17.6	2.4 ± 1
Free T ₄ ng/dL	1.92 ± 0.2	1.25 ± 0.3	0.24 ± 0.2	1.45 ± 0.4
T ₃ ng/mL	0.98 ± 0.15	0.73 ± 0.13	0.4 ± 0.2	1.07 ± 0.1

TSH, thyrotropin; T₃, triiodothyronine.

cant difference among CON and EUTHYR or sub HYPER groups was observed in the insulin tolerance test, the Kitt values were significantly lower in the HYPO group vs. CON. (3.5 ± 0.8 vs. 5.2 ± 0.7; $p < 0.01$ using the Kruskal-Wallis test, followed by the Dunn *post hoc* test to compare all the groups) indicating the presence of an insulin resistant state (Fig. 2). Interestingly, we were able to demonstrate in four patients, during the transition from the euthyroid to the hypothyroid state, the development of a significant degree of insulin resistance, as assessed by the short insulin tolerance test (Kitt: EUTHYR 4.7 ± 0.1 vs. HYPO 3.5 ± 0.8, $p < 0.05$, using paired *t* test).

The present observations contribute to the field of thyroid hormone action on the glucose homeostasis by confirming that in humans, overt acute hypothyroidism causes a state of insulin resistance characterized by a reduced glucose disposal.

Presently, most of the experimental data on the peripheral response of tissues to insulin action in hypothyroidism derive from animal and *ex vivo* experiments. Adipocytes and skeletal muscle of hypothyroid rats have been shown to be less responsive to insulin with regards to glucose metabolism (5,6,21,22). Furthermore, treatment of hypothyroid rats with T₃ increased glucose disposal in skeletal muscle (23). This is probably secondary to an increase in transcription of the GLUT₄ gene (24,25) mediated by a thyroid hormone respon-

FIG. 1. Plasma glucose levels (mean ± SD) measured before and after the intravenous (IV) administration (arrow) of 0.1 IU/kg of regular insulin to athyreotic subjects under mild hyperthyroid (empty circles, $n = 4$), euthyroid (filled triangles, $n = 4$), and hypothyroid conditions (empty squares, $n = 4$), as compared to healthy control subjects (filled squares, $n = 8$).

FIG. 2. Calculated K-index (upper panel) and decremental area under the curve (AUC, lower panel) in athyreotic patients under mild hyperthyroid (HYPER, dark columns, $n=4$), euthyroid (EUTHYR, light-shaded columns, $n=4$) and hypothyroid (HYPO, empty columns, $n=4$) conditions; data are presented as means $\pm$ SD; * $p < 0.05$; ** $p < 0.01$ (Kruskal-Wallis test followed by Dunn test) versus control subjects (CONTROLS, dotted columns, $n=8$). The data indicate a decrease in insulin-mediated glucose disposal in the HYPO group (see text for detail).

sive element. Moreover, local, tissue-specific regulation of thyroid hormones might also play a role in glucose homeostasis such as has been shown for mutations in the deiodinase 2 gene involved in the development of an insulin resistance state (26). Recently it has been suggested that hypothyroidism may be implicated in the changes of glucose homeostasis via an interference in the mitochondrial oxidative metabolism (27).

Our findings are in agreement with the previously reported association of hypothyroidism and insulin resistance in animal models (5,6) and to some of the studies in humans that analyzed whole body insulin sensitivity with different methodologies (7–12). Rochon *et al.* (7), using the hyperinsulinemic euglycemic clamp technique, demonstrated in six hypothyroid patients (two secondary to Hashimoto's thyroiditis and four to thyroid carcinoma) a decrease in the insulin-mediated glucose disposal that reverted upon treatment. Recently, Dimitriadis *et al.* (10) reached to similar conclusions in 11 female patients with primary hypothyroidism. A relevant finding of this study was that insulin resistance in hypothyroid muscle and adipose tissue can be, at

least in part, explained by an impairment in the ability of insulin to increase blood flow rate to those tissues. An improvement in insulin sensitivity after levothyroxine replacement therapy in 11 subjects with overt hypothyroidism due to Hashimoto's thyroiditis has been also demonstrated by Handisurya *et al.* (11). A proposed mechanism to explain their results was the elevation of circulating free fatty acids previously described by their group in the hypothyroid state.

By using the short IV insulin tolerance test we have demonstrated the presence of a significant state of insulin resistance in acute overt hypothyroidism resulting from thyroid hormone withdrawal. It is worth noting that a previous study in overt hypothyroid patients based on the homeostasis model assessment (HOMA-IR) (16) showed no association between hypothyroidism and insulin sensitivity. The discrepancies between our current findings and the studies based on the HOMA may be attributed to its relative low precision (28), and to the fact that HOMA measurements are obtained in a fasting state, thus reflecting mostly the hepatic gluconeogenesis status rather than the peripheral glucose disposal. In another study by Harris *et al.* (15), insulin-stimulated glucose disposal in the forearm of hypothyroid patients remained unchanged after treatment with levothyroxine. Insulin levels were higher during the clamp in the hypothyroid state compared to euthyroid state. This can be interpreted as a lower clearance rate of insulin while hypothyroid. In our study the use of a single pharmacologic bolus of insulin and the evaluation of a short-term endpoint (glucose at 15 minutes) might overcome the bias related to the delayed insulin catabolism observed in hypothyroid states (29), probably representing in this particular circumstances a better tool than the hyperinsulinemic euglycemic clamp where the goal is the achievement of sustained steady-state pharmacological plasma levels of insulin (30).

With regards to subclinical hyperthyroidism and insulin sensitivity, the available information is somewhat controversial. Two studies designed to investigate insulin sensitivity with HOMA in subclinical hyperthyroidism, stand out in the literature and differ in their results. Heemstra *et al.* (17) randomized 25 thyroid cancer patients to levothyroxine therapy at replacement or suppressive doses. This study failed to demonstrate any difference in insulin sensitivity between the two groups. On the other hand, Yavuz *et al.* (18) demonstrated a significant degree of insulin resistance in a cohort of 20 multinodular goiter patients that were treated for 6 months with levothyroxine at suppressive TSH doses. In our study we did not demonstrate any significant change in insulin sensitivity in our patients while in a subclinical hyperthyroid state when compared to the control group. This result has clinical relevance because, even if the use of levothyroxine therapy for goiter shrinkage is no longer considered a standard of care (31), TSH suppression represents a cornerstone in the treatment of differentiated thyroid cancer.

The major limitations of our study are the relative small number of study participants and the fact that the short IV insulin tolerance does not allow the evaluation of insulin secretion. Besides, this method relies on the assumption that insulin has the same pharmacokinetic characteristic in all groups. Nonetheless this rapid test correlates well with euglycemic hyperinsulinemic clamp (20), is quick and simple, and represents a valuable method to assess insulin-mediated glucose disposal.

In conclusion, our data indicate that the subclinical hyperthyroid state resulting from the administration of thyroid hormones at suppressive doses, does not affect insulin sensitivity in our group of patients. On the other hand, a short-term lack of thyroid hormone causes a state of insulin resistance characterized by a decreased insulin-mediated glucose disposal. These data further contribute to the understanding of the role of thyroid hormones action in the modulation of glucose metabolism.

Acknowledgments

This work was in part (FSC) supported by the Intramural Research Program Z01-DK047057-02 of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

References

- Dimitriadis G, Baker B, Marsh H, Mandarino L, Rizza R, Bergman R, Haymond M, Gerich J 1985 Effect of thyroid hormone excess on action, secretion and metabolism of insulin in humans. *Am J Physiol* **248**:593–601.
- Müller MJ, Paschen U, Seitz HJ 1983 Thyroid hormone regulation of glucose homeostasis in the miniature pig. *Endocrinology* **112**:2025–2031.
- Okajima F, Ui M 1979 Metabolism of glucose in hyper and hypothyroid rats in vivo. Glucose turnover values and futile cycle activities obtained with ^{14}C and ^3H labelled glucose. *Biochem J* **182**:565–575.
- McCulloch AJ, Nosadini R, Pernet A, Piniewska M, Cook DB, Clark F, Johnston DG, Alberti KGMM 1983 Glucose turnover and indices of recycling in thyrotoxicosis and primary thyroid failure. *Clin Sci* **64**:41–47.
- Czech MP, Malbon CC, Kerman K, Gitomer W, Pilch PF 1980 Effect of thyroid status on insulin action in rat adipocytes and skeletal muscle. *J Clin Invest* **66**:574–582.
- Dimitriadis G, Parry-Billings M, Bevan S, Leighton B, Krause U, Piva T, Tegos K, Challiss RA, Wegener G, Newsholme EA 1997 The effects of insulin on transport and metabolism of glucose in skeletal muscle from hypothyroid rats. *Eur J Clin Invest* **27**:475–483.
- Rochon C, Tauveron I, Dejax C, Benoits P, Capitan P, Fabricio A, Berry C, Champedon C, Thieblot P, Grizard J 2003 Response of glucose disposal to hyperinsulinemia in human hypothyroidism and hyperthyroidism. *Clin Sci* **104**:7–15.
- Andreani D, Menzinger G, Fallucca F, Aliberti G, Tamburano G, Cassano C 1970 Insulin levels in thyrotoxicosis and primary myxoedema: response to intravenous glucose and glucagon. *Diabetologia* **6**:1–7.
- Pedersen O, Richelsen B, Bak J, Arnfred J, Weeke J, Schmitz O 1988 Characterization of the insulin resistance of glucose utilization in adipocytes from patients with hyper- and hypothyroidism. *Acta Endocrinol* **119**:228–234.
- Dimitriadis G, Mitrou P, Lambadiari V, Boutati E, Maratou E, Panagiotakos DB, Koukkou E, Tzanela M, Thalassinou N, Raptis SA 2006 Insulin action in adipose tissue and muscle in hypothyroidism. *J Clin Endocrinol Metab* **91**:4930–4937.
- Handisurya A, Pacini G, Tura A, Gessl A, Kautzky-Willer A 2008 Effects of thyroxine replacement therapy on glucose metabolism in subjects with subclinical and overt hypothyroidism. *Clin Endocrinol (Oxf)*. Apr 21 [Epub ahead of print].
- Stanická S, Vondra K, Pelikánová T, Vlcek P, Hill M, Zamrazil V 2005 Insulin sensitivity and counter-regulatory hormones in hypothyroidism and during thyroid hormone replacement therapy. *Clin Chem Lab Med* **43**:715–720.
- Lamberg BA 1965 Glucose metabolism in thyroid disease. *Acta Med Scand* **178**:351–362.
- Jackson IMD, Prentice CRM, McKiddie MT 1970 The effect of hypothyroidism on glucose tolerance and insulin metabolism. *J Endocrinol* **47**:257–258.
- Harris PE, Walker M, Clark F, Home PD, Alberti KGMM 1993 Forearm muscle metabolism in primary hypothyroidism. *Eur J Clin Invest* **23**:585–588.
- Owecki M, Nikisch E, Sowiński J 2006 Hypothyroidism has no impact on insulin sensitivity assessed with HOMA-IR in totally thyroidectomized patients. *Acta Clin Belg* **61**:69–73.
- Heemstra KA, Smit JW, Eustatia-Rutten CF, Heijboer AC, Frölich M, Romijn JA, Corssmit EP 2006 Glucose tolerance and lipid profile in longterm exogenous subclinical hyperthyroidism and the effects of restoration of euthyroidism, a randomised controlled trial. *Clin Endocrinol (Oxf)* **65**:737–744.
- Yavuz DG, Yüksel M, Deyneli O, Ozen Y, Aydin H, Akalin S 2004 Association of serum paraoxonase activity with insulin sensitivity and oxidative stress in hyperthyroid and TSH-suppressed nodular goitre patients. *Clin Endocrinol (Oxf)* **61**:515–521.
- Bonora E, Targher G, Formentini G, Calcaterra F, Lombardi F, Marinin L, Zenari F, Saggiani M, Poli S, Perbellini A, Raffaelli A, Gemma L, Santi L, Bonadonna RC, Muggeo M 2004 The metabolic syndrome is an independent predictor of cardiovascular disease in type 2 diabetes subjects. *Diabet Med* **21**:52–58.
- Bonora E, Moghetti P, Zancanaro C, Cigolini M, Querena M, Cacciatori V, Corgnati A, Muggeo M 1989 Estimates of in vivo insulin action in man: comparison of insulin tolerance tests with euglycemic clamp studies. *J Clin Endocrinol Metab* **68**:374–378.
- Dubanievicz A, Kaciuba-Uscilko H, Nazar K, Budohoski L 1989 Sensitivity of the soleus muscle to insulin in resting and exercising rats with experimental hypo- and hyperthyroidism. *Biochem J* **263**:243–247.
- Shoji, S 1991 Effect of thyroxine on basal and insulin-stimulated glucose uptake by fast and slow skeletal muscles of rats. *Comp Biochem Physiol* **100**:343–345.
- Weinstein SP, Watts J, Haber RS 1991 Thyroid hormone increases muscle/fat glucose transporter gene expression in rat skeletal muscle. *Endocrinology* **129**:455–464.
- Torrance CJ, Usala SJ, Pessin JE, Dohm GL 1997 Characterization of a low affinity thyroid hormone receptor binding site within the rat GLUT₄ gene promoter. *Endocrinology* **138**:1215–1223.
- Torrance CJ, Devente JE, Jones JP, Dohm GL 1997 Effects of thyroid hormone on GLUT₄ glucose transporter gene expression and NIDDM in rats. *Endocrinology* **138**:1204–1214.
- Chidakel A, Mentuccia D, Celi FS 2005 Peripheral metabolism of thyroid hormone and glucose homeostasis. *Thyroid* **15**:899–903.
- Crunkhorn S, Patti ME 2008 Thyroid economy—regulation, cell biology, thyroid hormone metabolism and action: the special edition: metabolic effects of thyroid hormones. links between thyroid hormone action, oxidative metabolism, and diabetes risk? *Thyroid* **18**:227–237.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC 1985 Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**:412–419.

29. Elgee NJ, Williams RH 1955 Effects of thyroid function on insulin-¹³¹I degradation. *Am J Physiol* **180**:13–15.
30. De Fronzo RA, Tobin JD, Andres R 1979 Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* **237**:214–223.
31. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Sherman SI, Tuttle RM 2006 The American Thyroid Association Guidelines Taskforce. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* **16**:109–142.

Address reprint requests to:

Gabriela Brenta, M.D.

Department of Endocrinology and Metabolism

Cesar Milstein Hospital

La Rioja 951, Cap Fed

Buenos Aires 1221

Argentina

E-mail: gbrenta@gmail.com